PATENT
Docket No. 104914.127

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor:	Leiden *et al.*
Serial No.:	09/473,830
Filing Date:	December 28, 1999
For:	Efficient and Stable *In Vivo* Gene Transfer to Cardiomyocytes Using Recombinant Adeno-Associated Virus Vectors
Examiner:	Chen, Shin-Lin
Art Unit:	1632/1633

Assistant Commissioner for Patents
Washington D.C. 20231

DECLARATION OF MICHAEL S. PARMACEK, M.D.

I, Michael S. Parmacek, hereby declare that:

1. I obtained a B.S. degree in Biology/Psychology from Tufts University, Boston, Massachusetts in 1977; and an M.D. degree from Northwestern University Medical School, Chicago, Illinois in 1981.

2. I was employed as an Intern and Resident at the University of Michigan Medical School from 1981-1984; as a Fellow for Cardiovascular Disease at Northwestern University Medical School from 1984-1987; as a Postdoctoral Fellow at the Howard Hughes Medical Institute, University of Michigan from 1987-1989; as an Instructor from 1987-1990 and as an Assistant Professor from 1990-1992 in the Department of Internal

Medicine, Section of Cardiology, University of Michigan Medical School; and as an Assistant Professor from 1992-1997 and as an Associate Professor from 1997-1998 in the Department of Medicine, Section of Cardiology and Committees on Cancer Biology and Cell Physiology, University of Chicago.

3. I am presently the Herbert C. Rorer Professor of Medical Sciences in the Department of Medical Sciences at University of Pennsylvania School of Medicine, where I have been employed since 1998. A copy of my *curriculum vitae* is attached as Exhibit 1.

4. I have over 17 years experience in conducting cardiovascular research, including studies relating to gene transfer and expression in the cardiovascular system.

5. I have served as a paid consultant for Applicant's exclusive licensee.

6. I have read and understand U.S. Serial No. 09/473,830, filed December 28, 1999 (hereinafter "the Leiden application"). I have also read and understand the Office Actions dated August 29, 2001 and March 7, 2002.

7. I have been told and understand that the claims of the Leiden application have been rejected for lack of enablement.

Claims

8. I have been informed that the sole pending independent claim in the Leiden application (Claim 24) is as set forth below:

> 24. A method of introducing a nucleic acid encoding a desired molecule into cardiomyocytes which comprises:
>
> infusing a recombinant adeno-associated virus (AAV) vector into a coronary artery or a coronary sinus for a time and in an amount sufficient to stably and efficiently transduce cardiomyocytes perfused through said artery or said sinus, wherein said AAV vector comprises at least one nucleic acid operably linked to a control region, said nucleic acid encoding said desired molecule.

9. It is my understanding that Claim 24 is directed to a method of introducing a nucleic acid encoding a desired molecule into cardiomyocytes. As set forth in that claim, this method is accomplished by infusion of a recombinant adeno-associated virus (AAV) into a coronary artery or coronary sinus. The recombinant AAV contains at least one nucleic acid, which encodes a desired molecule (i.e., the transgene), linked to a control region operable in cardiomyocytes. The recombinant AAV is infused in a sufficient amount and for a sufficient time to stably and efficiently transduce the cardiomyocytes.

Evidence

10. The Leiden application contains data demonstrating that the infusion of a recombinant AAV vector encoding the marker gene β-galactosidase under the control of the cytomegalovirus (CMV) promoter into the coronary artery of a mouse resulted in the

expression of that gene by greater than 50% of cardiomyocytes in the perfused myocardium at eight weeks post-perfusion (Example, pp. 10-11).

11. As of December 28, 1998, the priority date of the Leiden application, the construction of a recombinant AAV vector encoding a desired molecule operably linked to a control region would have been routine. Gnatenko *et al.* (1997), *J. Invest. Med.* 45:87-98; Kessler *et al.* (1996), *Proc. Natl. Acad. Sci. USA* 93:14082-14087; Kotin (1994), *Hum. Gene Ther.* 5:793-801 (particularly Table 1); Lebkowski *et al.* (1988), *Mol. Cell. Biol.* 8:3988-3996; Phillips *et al.* (1997), *Hypertension* 29:374-380; Rolling *et al.* (1995), *Mol. Biotech.* 3:9-15.

12. As of December 1998, many proteins and antisense RNA sequences useful for treating cardiovascular conditions were known, and cloned DNA encoding these proteins and RNA sequences was readily available. For example, cloned DNA encoding many angiogenic factors, including acidic FGF, basic FGF, FGF-5, platelet-derived growth factor (PDGF), angiogenin, and vascular endothelial growth factor (VEGF), was available. Connolly *et al.* (1991), *J. Cell. Biochem.* 47:219-223; Crumley *et al.* (1990), *Biochem. Biophys. Res. Commun.* 171:7-13; Folkman *et al.* (1987), *Science* 235:442-447; Kurachi *et al.* (1985), *Biochemistry* 24:5494-5499; Kurokawa *et al.* (1987), *FEBS Lett.* 213:189-194; Leung *et al.* (1989), *Science* 246:1306-1309; Schaper *et al.* (1991), *Basic Res. Cardiol.* 86(Suppl. 2):51-56.

13. The Leiden application contains sufficient guidance for the preparation of high titers of AAV vectors and determination of the amount of AAV vector needed to transduce cardiomyocytes in a given animal. The Leiden application teaches that the preferred amount of infused AAV vector may range from about 10^5 infectious units (IU) AAV per gram body weight of the subject animal to about 10^9 IU AAV per gram body weight, preferably from about 10^6 IU AAV per gram body weight to about 10^8 IU AAV per gram body weight, and most preferably about 5×10^7 IU AAV per gram body weight to about 6×10^7 IU AAV per gram body weight. In the Example in the Leiden application, a mouse heart is perfused with 1.5×10^9 IU AAV per gram body weight.

14. Before December 1998, several groups had demonstrated that transduction of mouse or rat cardiomyocytes *in vitro* or *ex vivo* using rAAV vectors encoding the marker gene β-galactosidase led to detectable expression of β-galactosidase. Kessler *et al.* (1995), *Circulation* 92:I-296 (*in vitro*); Kourtis *et al.* (1995), *Modern Pathology* 8:Abstract No. 178 (*in vitro*); Maeda *et al.* (1998), *J. Moll. Cell. Cardiol.* 30:1341-1348 (*in vitro* and *ex vivo*).

15. Well before December 1998, the marker gene β-galactosidase had been expressed *in vitro* in growth-arrested human kidney cells and fibroblasts using rAAV vectors. Podsakoff *et al.* (1994), *J. Virol.* 68:5656-5666.

16. Before December 1998, it had been demonstrated that the *in vivo* intracardiac injection of rAAV encoding the marker gene β-galactosidase in rats resulted in the expression of β-galactosidase in myocardial cells for up to 2 months post-injection. Kaplitt *et al.* (1996), *Ann. Thorac. Surg.* 62:1669-1676.

17. Before December 1998, several groups had demonstrated the therapeutic effectiveness of *in vivo* rAAV vector transduction in rodent cells. The intraventricular or intracardiac injection of rAAV encoding angiotensinogen receptor (AT_1-R) antisense RNA reduced blood pressure and slowed the development of hypertension in rats. Phillips *et al.* (1997), *Hypertension* 29:374-380. Therapeutic concentrations of the gene encoding Factor IX had been expressed in mouse hepatocytes using rAAV vectors infused into the portal vein. Snyder *et al.* (1997), *Nature Genet.* 16:270-276. Therefore, one of skill in the art would have understood that successful transduction with therapeutic molecules would have therapeutic benefit.

18. Before December 1998, rAAV vectors had been used *in vivo* to express genes encoding various markers and therapeutic proteins in the organs of animals other than mice or rats. For example, the alkaline phosphatase (AP) reporter gene was expressed in the carotid adventitia of cynomolgus monkeys using rAAV vectors infused or injected into the carotid artery. Lynch *et al.* (1997) Circ. Res. 80:497-505. Another study demonstrated sustained expression of human Factor IX in hemophilic dogs following

intramuscular injection of rAAV vectors encoding the clotting factor. Monahan *et al.* (1998), *Gene Ther.* 5:40-49. Furthermore, the gene encoding amino acid decarboxylase (AADC) had been expressed at levels sufficient to partially correct dopamine deficiencies in the caudate of green monkeys using injected rAAV vectors. During *et al.* (1998), *Gene Ther.* 5:820-827. Finally, the gene encoding cystic fibrosis transmembrane conductance regulator (CFTR) had been expressed in the bronchial epithelium of rabbits and rhesus monkeys using aspirated rAAV vectors. Flotte *et al.* (1993), *Proc. Natl. Acad. Sci. USA* 90:10613-10617 and Conrad *et al.* (1996), *Gene Ther.* 3:658-668, respectively. The levels of CFTR RNA expressed in the rhesus monkeys were similar to endogenous levels in humans. Conrad *et al.* (1996), *Gene Ther.* 3:658-668.

19. A phase I human trial of rAAV-CFTR gene vectors in human cystic fibrosis subjects was initiated in August 1999, based at least in part on several of the studies cited in the preceding paragraph. Flotte *et al.* (1996), *Hum. Gene Ther.* 7:1145-1159 (particularly Section 3.4) and "Phase I Randomized Study of Adeno-Associated Virus-CFTR Vector in Patients with Cystic Fibrosis," www.clinicaltrials.gov/ct/gui/c/w1r/show/NCT00004533?order=1&JservSessionIdzone_ct=xnrwsoycu1 (downloaded from website on June 13, 2002).

20. In December 1998, it was well known that rAAVs were particularly suited for use as gene transfer vectors given their stable integration into the host cell's genome,

long-term transgene expression, lack of immunogenicity, and ability to transduce non-dividing cells. Flotte *et al.* (1996), *Hum. Gene Ther.* 7:1145-1159 (particularly Section 3.2); Gnatenko *et al.* (1997), *J. Invest. Med.* 45:87-98 (particularly p. 88, col. 1, first full paragraph); Maeda *et al.* (1997), *Cardio. Res.* 35:514-521 (particularly pp. 514-515, bridging paragraph); Phillips *et al.* (1997), *Hypertension* 29:374-380 (particularly p. 379, col. 2, first full paragraph).

Level of Skill in the Art

21. In my opinion, in December 1998, one of ordinary skill in the art of gene transfer and expression into the cardiovascular system would have had a medical degree (M.D.) and/or a doctorate degree (Ph.D.) in an area such as cardiology, anatomy, molecular biology, or in a related field such as physiology, and at least several years research and/or clinical experience following completion of the advanced degree. In addition, those of ordinary skill in the art would also have had at least one or more years of actual experience in the field of gene transfer.

Utilities

22. Based upon the entire disclosure of the application, I believe that the utilities of the disclosed methods of transducing cardiomyocytes would be immediately apparent and recognized as well-established by one of ordinary skill in the art at the time the application was filed. Such utilities include, but are not necessarily limited to, transducing human or non-human animal hearts *ex vivo* to create organ models for human

cardiovascular disease, transducing non-human animal hearts *in vivo* to create animal models for human cardiovascular disease, and gene therapy of human cardiovascular disease.

Conclusions

23. Based on the information in paragraphs 11-13, it would have been a matter of routine experimentation at the time of the invention to construct a recombinant AAV vector encoding a desired molecule operably linked to a control region for transduction of cardiomyocytes. The mechanics of selecting vectors, genes, and promoters and combining them into expression constructs were well known at that time. Moreover, as evidenced by paragraph 12, cloned DNA encoding proteins and antisense RNA useful for treating cardiovascular conditions was available for incorporation into these expression constructs. Hence, one of skill in the art at the time of the invention could have selected a combination of rAAV vectors, promoters and genes suitable to produce expression of a desired molecule in cardiomyocytes.

24. As shown in paragraphs 10 and 14-17, effective transduction of various rodent and human cells using rAAV vectors encoding marker and therapeutic genes had been demonstrated at the time of the invention.

25. As shown in paragraph 18, genes encoding markers and therapeutic proteins had been expressed in various organs of animals other than rodents using rAAV vectors administered by some of the same routes known for transduction of rodent cells. For example, canine and primate cells expressed therapeutic genes, Factor IX and AADC, respectively, following local injection of rAAV vectors.

26. As shown in paragraphs 17-19, rAAV vectors had been used to express therapeutically-effective amounts of several genes in the liver, brain, vasculature, and lungs of various non-human animals at the time of the invention. A phase I trial to determine the therapeutic effectiveness of rAAV-CFTR gene vectors in human subjects had also been initiated.

27. In my opinion, the Leiden application and the state of the art enabled one of skill in the art to practice the claimed invention in December 1998. Based on such teaching and guidance in the Leiden application, along with the state of the art described herein with respect to molecular biological techniques and *in vivo* gene transfer, it was merely a matter of routine experimentation to construct a recombinant AAV vector encoding a desired molecule, deliver it to an animal by infusion into a coronary artery or coronary sinus, and thereby transduce cardiomyocytes.

28. Since the filing of the Leiden application, others in the field have done nothing more than follow the teaching of the Leiden application and successfully

transduce mouse cardiomyocytes using rAAV vectors encoding the marker gene β-galactosidase under the control of the CMV promoter. Svensson *et al.* (1999), *Circulation* 99:201-205.

29. Accordingly, it is my opinion that the Leiden application and the state of art at the time provided one of skill in the art sufficient guidance to make and use the full scope of the claimed invention without requiring significant or undue experimentation, that is, with a reasonable expectation of success.

30. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on knowledge and belief are believed to be true and further that these statements are made with the knowledge that willful false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the U.S. Code and that such willful false statements may jeopardize the validity of U.S. Serial No. 09/473,830 or any patent issuing thereon.

Date: _____ _____

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Date: 7-3-02

Michael S. Parmacek